DIETARY INFLUENCE ON THE GROWTH AND FECUNDITY OF *ONYCHIURUS JUSTI* (DENIS), (*ONYCHIURIDAE* : *COLLEMBOLA*) (1)

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INTRODUCTION

Many investigations involving food preference, feeding biology, and growth of Collembola have been carried out. Reviews of the literature dealing with collembolan ecology an biology can be obtained from POOLE (1959), CHRISTIANSEN (1964), SCHALLE (1970) and BUTCHER, SNIDER and SNIDER (in press). Recent life history studies by GREEN (1964), HALE (1965), SHARMA (1967 *a*, *b*), THIBAUD (1967, 1968 *b*, *c*), ASHRAF (1969) and food preference studies by SINGH (1969) have greatly expanded our knowledge of collembolan bionomics.

Most laboratory studies have attempted to simulate field conditions or at least provide natural foods while manipulating temperature and humidity factors. As a result of most of those studies it is common to use foods such as fungi, yeasts, spores, leaf material and detritus. The influence of specific diets on growth has been, for the most part, a neglected area of investigation. KNIGHT and ANGEL (1967) cultured *Tomocerus flavescens* (Tullberg) on fungi found in forested areas and classified food preference by gut content analysis. However, no studies were pursued to determine the effects of those foods on growth. While studying cellulose decomposition by several species of Collembola, TORNE (1967) showed that the population dynamics of the species were regulated by microbial influence on food material in the gut. The effects on fecundity and general biology of *Hypogastrura manubrialis* (Tullberg) by feeding yeast, liver

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mushroom, banana, algae and blood agar base were noted by VAIL (1965). He demonstrated that *H. manubrialis* was a general feeder, but does best on yeast.

In our laboratory at Michigan State University, we have been engaged in mass rearing attempts of many collembolan species. Up to the time this investigation commenced, we used brewer' syeast as our basic food source in all the cultures. It became increasingly evident that not all species survive well on yeast. Some cultures died out after a brief period of growth, others would not reproduce. It was soon apparent that readily available and uniform food sources needed to be tried.

A premiminary testing of food materials that might influence growth and fecundity other than fungi was necessary for our research. Consequently, the present study was undertaken while the author was engaged in observing the bionomics of *Onychiurus justi* (Denis).

MATERIALS AND METHODS

Laboratory cultures of *Onychiurus justi*, obtained from a Michigan mushroom house, were maintained at 70°F (21.11°C) and used as a source of eggs. The eggs were transferred from the stock cultures with a needle to small plastic containers (35 mm in diameter by 20 mm high) with plaster-charcoal substrates (SNIDER, SHADDY and BUTCHER, 1969). The culture containers were moistened with distilled water at daily intervals to maintain them as close to 100 p. 100 relative humidity as possible. After an incubation period of 14 days at 70°F, the eggs hatched. The juveniles were floated on water and transferred with the hairs of a brush in groups of 10 to fresh containers. In all, 77 containers with 10 individuals per container (770 individuals) were set up.

The diets used consisted of the brewer' syeast commonly used in many investigations, and two commercially prepared foods designated here as diet « A » and diet «B »(1). A control was set up with no food at all. Diet «A » has the following ingredients : « fish meal, fish roe meal, fish liver and glandular meal, tropical crayfish meal, insect larvae meal, dehydrated kelp meal, mussel meal, brine shrimp meal, wheat germ and cod liver oil. » The ingredients of diet « B » are as follows : « egg yolk, 03 p. 100 cod liver oil, tapioca, wheat and corn flour and corn starch. » Table 1 indicates the guaranteed analysis by the manufacturer for both diets « A » and « B ».

TABLE 1

Constituents of diets « A » and « B » as provided by the manufacturer

	DIET "A"	DIET "B"
CRUDE PROTEIN	NOT LESS THAN 45%	NOT LESS THAN 3.4%
CRUDE FAT	NOT LESS THAN 4%	NOT LESS THAN 0.9%
CRUDE FIBER	NOT MORE THAN 9%	NOT MORE THAN 0.5%
ASH	NOT MORE THAN 15%	NOT MORE THAN 10%

Small amounts of yeast, diet « A » and diet «B» were added to the cultures each day as required. Food not eaten within 24 hours was removed. If the food showed signs of contamination, it was removed and replaced. As the cultures were examined each day during the 100 days the experiment was in progress, it was a simple matter to scrape the substrate surface to keep it relatively free of moulds.

(1) Diets « A » and « B » were manufactured by Hartz Mountain Products Corp., New York, N. Y. 10003.

Ultimately, then, thee were four runs in the experiment designated as yeast (15 replicates), diet « A » (25 replicates), diet « B » (30 replicates) and control (7 replicates). The number of individuals observed is indicated in table 2.

TABLE 2

Number of individuals of Onychiurus justi (DENIS) *used per diet*

DIET "A"
(250)

DIET "B"
(300)

YEAST
(BREWERS)
(150)

CONTROL
(NO FOOD)
(70)

TOTAL INDIVIDUALS = 770

As deaths occurred, the bodies were removed and their number noted. Ecdysis was recorded daily by counting and removing the exuviae. The number of eggs laid were recorded by batch. When the eggs hatched, the juveniles were counted and removed. All individuals still living at the end of the experiment were killed and preserved in hot 85 p. 100 ethanol for later measurement with an ocular micrometer.

RESULTS

As previously mentioned, the eggs took 14 days to hatch at 70°F (21.11°C). The following table 3 shows the duration of instars in days for each of the four runs.

TABLE 3

Instar duration in days (average of all replicates per diet)

Instar	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Control ..	6.9	8.4	10.6	8.3	8.4	9.9	8·4	7.9	9.6	7.4	7.6	8.4	7.8	8.4	7.0
Diet « B »	7.3	7.0	7.1	7.8	8.4	9.3	8.9	8.0	9.0	8.8	9.2	8.8	8.6	9.2	9.1
Diet « A »	6.0	5.8	5.5	5.8	7.2	8.7	8.8	8.5	8.0	7.0	77.6	7.5	8.4	7.5	7.6
Yeast ...	5.9	6.6	5.8	5.5	6.9	6.9	6.9	7.6	7.7	7.3	7.5	6.7	7.1	7.0	7.2

There is an obvious similarity of effect between the individuals reared on yeast and those reared on diet « A ». Both resulted in a more rapid rate of growth as indicated by instar duration. After the 5th instar, there is a marked similarity between the control

and diet « B » in instar duration. This may be the result of bacterial build-up in the substrate of the control cultures. Control specimens were seen eating the substrate and the black contents of the gut were clearly visible through their body walls.

The similarities in rate of growth can also be graphically shown by plotting the number of days to the beginning of each instar, starting from the day of hatching.

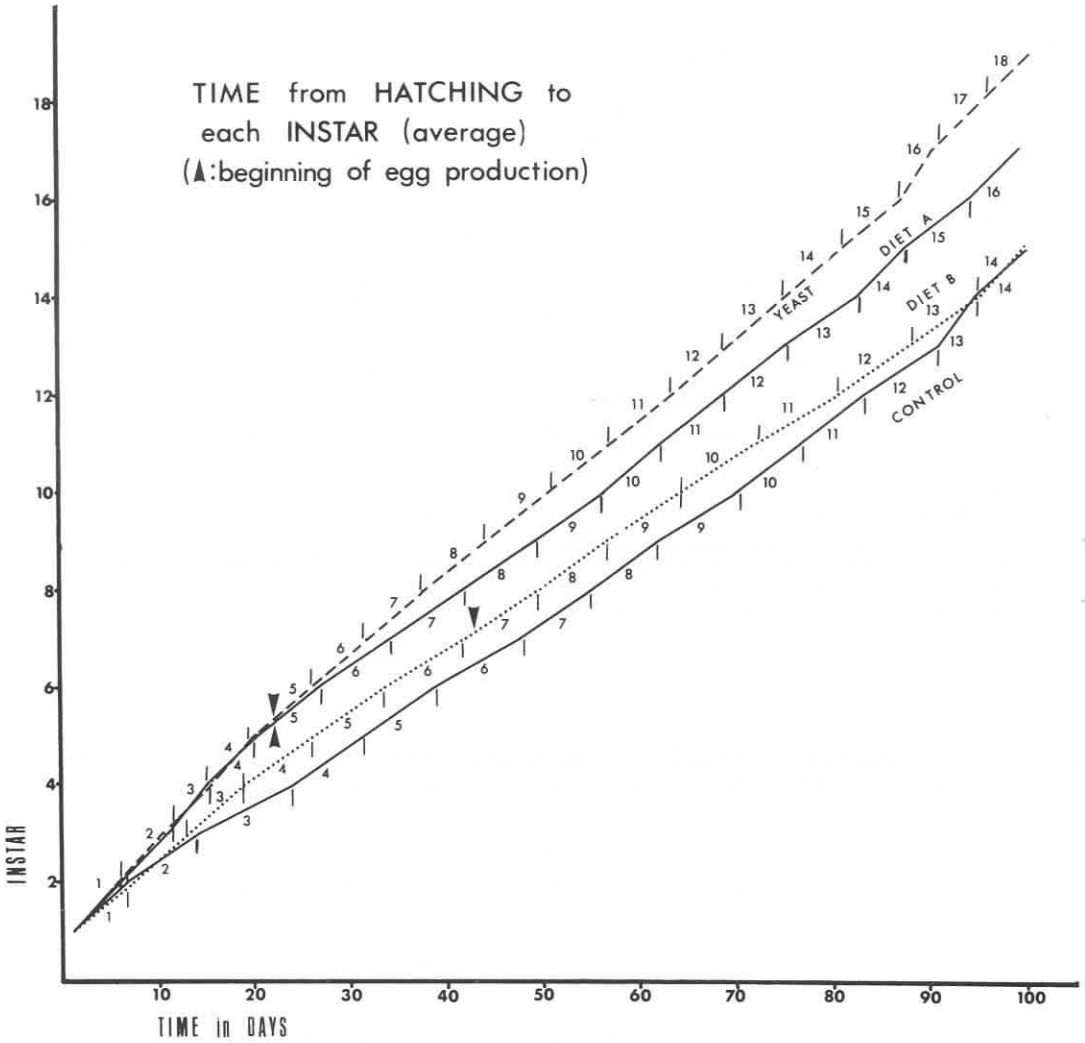

FIG. 1. — *Time from hatching to each instar* (average)
(▼ : beginning of egg production)
Temps de l'éclosion à chaque stade (moyenne)
(▼ : début de la ponte)

Figure 1 clearly shows the diversity of growth rate between yeast, diet « A », control, and diet « B ». Here the lag in instar duration shows up after the first two instars.

Mortality can be inferred from figures for percent survival of the total population. The following table 4 shows the survival of each run after 25, 50, 75 and 100 days.

TABLE 4

Influence of various diets on the survival of Onychiurus justi (DENIS)

	Age in days			
	25	50	75	100
Control	78,6	61,4	34,3	27,1
Diet « B » ...	87,6	77,0	73,3	71,3
Diet « A » ...	96,0	92,4	78,8	61,6
Yeast	89,3	83,3	67,3	56,6

When the percent survival is graphed on a daily basis (fig. 2), one can see the expected downward slope of the control which reaches 50 p. 100 after about 55 days. It is interesting to note that under diet « B », not the best diet for growth, survival stabilize after approximately 50 days and mortality is at a mnimum. On the other hand, both yeast and diet « A » produce similar trends of survival, gradually sloping downward between 50 and 100 days.

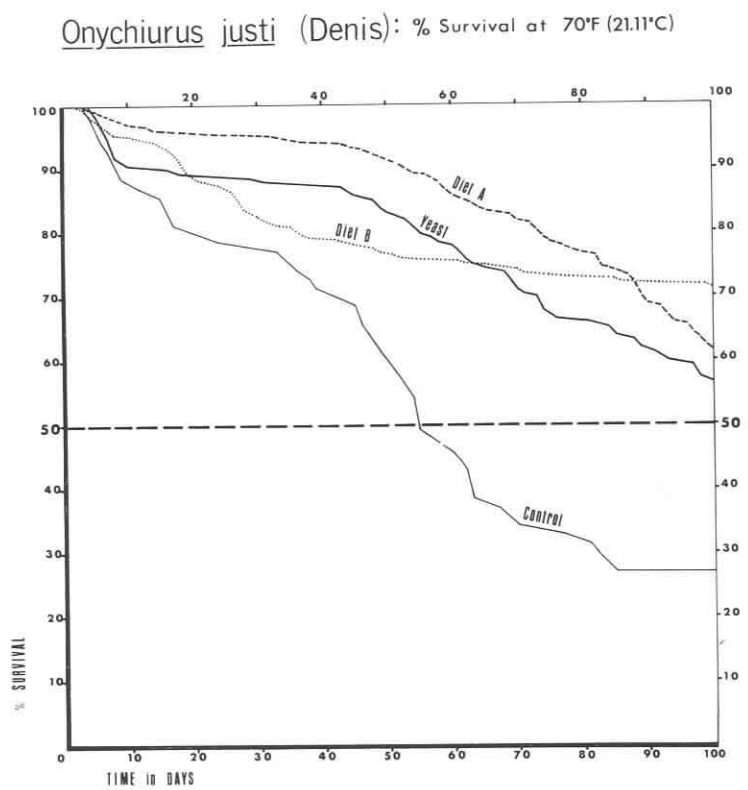

FIG. 2. — Onychiurus justi (DENIS) : *p. 100 survival at 70°F* (21.11°C)
Onychiurus justi DENIS : *povrcentage de survie à 70°F 21.11°C*

Egg production was recorded for each of the four runs. The control did not produce eggs at any time during the 100 day period. However, egg production was observed in the other three runs. Since the culture containers were each started with 10 individuals, it was impossible to consider individual egg production on a per female basis. Therefore the number of eggs produced each week during 100 days by the average number of individuals surviving each week was calculated. It must be noted that this figure includes the males present in each run. From casual observations by the author, the ratio of males to females in laboratory populations appears quite low. Figure 3 indicates egg production according to the formula :

$$\text{egg production per individual} = \frac{n \text{ number of eggs/week}}{\text{ave. number individuals/week}}.$$

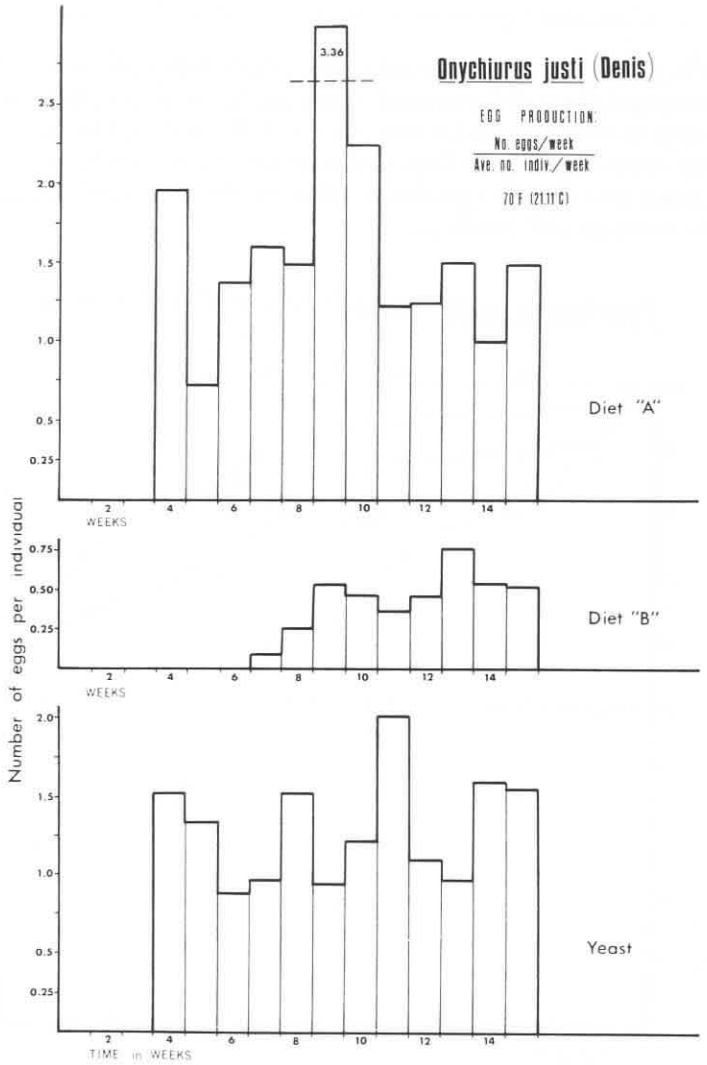

FIG. 3. — Onychiurus justi (DENIS) : *egg production*
Onychiurus justi DENIS : *production des œufs*

Note that figure 3 shows no periodicity of egg production. When observations were made of paired individuals cultured for a year, in some cases 12 egg laying periods were observed. Most commonly, 4-5 periods were noted between the 5th and 15th instar. This information will be presented elsewhere.

Fecundity appeared highest among individuals cultured on diet « A ». Yeast cultured individuals were next, followed by those fed diet « B ». Egg laying started during the 5th instar, which occurred the fourth week after hatching in both the diet « A » and yeast reared runs. It is interesting to note that diet « B »-fed individuals did not begin egg laying until the seventh week after hatching when the seventh instar had been reached. Also of importance is the relatively low egg production by diet « B »-fed individuals. While diet « A »-fed individuals layed as many as 50 eggs per clutch and yeast fed individuals nearly the same, those fed diet « B » neuer layed beyond 17 eggs per clutch. On the average, the diet « B » run produced 5-8 eggs per clutch.

Egg viability was lowest in the run fed on diet « A ». Of the 2991 eggs laid that were observed, 2 359 or 78 p. 100 hatched. In the case of diet « B », of 462 eggs observed, 476 were viable, or 84 p. 100. Yeast showed 81 p. 100 viability for 1 373 eggs observed with 1 114 hatching. The juveniles that hatched were not reared and no figures are available on their survival.

At the end of the 100-day period, the individuals of each run were killed and slide mounted for measurements. Not all runs were killed at the same time. Some replicates were still producing eggs, and it was necessary to allow some clutches of eggs to hatch before killing. The following table (table 5) indicates the average size of individuals killed at various time periods after 100 days.

TABLE 5

Mean overall lengths (in microns), *at the end of various time periods, of* Onychiurus justi (DENIS) *reared on different diets*

DAY REPLICATES KILLED	DIET "A"	DIET "B"	YEAST	CONTROL
105	(12)* 1722.4	(62) 1280.5	(18) 1632.6	---
107	(18) 1489.1	---	(22) 1757.1	---
108	---	(55) 1349.6	---	(5) 870.3
112	(54) 1583.2	---	---	(1) 1066.8
113	(16) 1602.0	(33) 1257.2	---	---
114	(24) 1852.9	---	(24) 1800.8	(7) 840.7
145	---	(16) 1254.3	(32) 1659.6	---

* NUMBER CONTRIBUTING TO THE MEAN

When the egg hatches, the 1st instar juvenile is on the average 445 microns in length. The figures in table 5 indicate that the control grows very little throughout the experiment. In fact, their size after 100 days is between 840-1 066 microns or the

average size for 3rd instar juveniles fed yeast. They were actually in the 14th instar when they were killed. They underwent ecdysis even though no appreciable size was attained after reaching the 3rd instar size.

Diet « A » and yeast produced individuals of approximately equal size after 100 days. Diet « B »-fed idividuals were small by comparison. Their size compares with yeast-fed 4th to 5th instar individuals which were raised at the same temperature. The figures of Table 5 indicate size fluctuations between 105 and 145 days. This condition seems to be quite normal for individuals after the 7th instar. Data gathered by the author in another experiment (to be published elsewhere) indicates that after the 7th instar, size (length) fluctuates. That is, there may be decrease in length instead of an increase. This increase and decrease rhythm continues until the animals reach senility, when they continually decrease a small amount with each succeeding instar until death.

DISCUSSION

The results of this preliminary study indicate that the type and quality of diet can influence growth and fecundity of *Onychiurus justi*. The amount of protein and fat in diets « A » and « B » probably are, to an extent, responsible for these differences. It should be noted that no biochemical analysis has yet been performed on any of the diets. Therefore, it cannot be stated with accuracy whether a specific protein(s) was responsible for the resultant growth and fecundity patterns.

Considering the differences in protein content as a whole, we can rank the three food runs on the basis of high protein to low : diet « A » (45 p. 100 protein), yeast (approx. 40 p. 100 protein), diet « B » (3.4 p. 100 protein). Referring to figure 1 showing rate of growth ann Table 3 indicating instar duration, differences are apparent. Diet « B » has the longest instar durations, followed by diet « A », and yeast the shortest. Even though yeast has somewhat less protein content than diet « A », the yeast's protein may be easier to assimilate by the Collembola.

Survival, as indicated in figure 2 and Table 4, presents some interesting factors. After an initial decline up to 35 days, diet « B »-fed individuals even out, or stabilize through the rest of the 100-day observation period. Meanwhile, both diet « A »-fed and yeast-fed individuals begin steady decline after 42 days. These survival patterns may be due to a multiplicity of factors. Perhaps diet « B »-fed individuals adjusted to the low protein food, with higher assimilation efficiency under stress conditions. It may be that the animals reacted much like dermestid larvae when placed on starvation or near starvation diets (D. BECK, in litt).

One of the most interesting developments was the difference in egg production. As expected, the controls (starved) produced no eggs. Diet « B », low protein, produced few eggs compared to diet « A »-fed and yeast-fed individuals. Moreover, diet « B »-fed cultures lagged in egg production until the 7th week of the experiment. Diet « A »-fed cultures had the highest rate or egg production followed by yeast-fed cultures. But eggs in diet « B »-fed cultures showed the highest viability, followed by yeast-fed and diet « A »-fed cultures.

Finally, there is a marked difference in size between the four runs after 100 days on their respective diets. For overall size, the diet « A »-fed individuals were largest followed by yeast-fed, diet « B »-fed and the control.

As has previously been mentioned, every attempt was made to keep the cultures as clean as possible throughout the 100 days of the experiment. Food was not left in the cultures for more than 24 hours, after which it was replaced by fresh. Fungal growth was kept at a minimum by scraping the substrate whenever the food was replaced. However, no control of bacteria was feasible ; and it is possible that contamination may have supplemented the diets. In the case of the control, it is very probable that contamination made it possible for some of the individuals to survive the 100 days of the experiment. But it is still striking that they survived at all and continued to moult without appreciable size increase at regular intervals.

From the data collected during this experiment, it becomes increasingly evident that food quality as well as temperature and humidity play an important role in collembolan growth and fecundity. Consideration of the type of food and its availability must be taken into account when considering biomass and age structure of collembolan populations in the laboratory and the field. As I have pointed out, the size of individuals, however advanced in age, bears no relation to the instar they are in, if maintained on an insufficient diet. For instance, the control and diet « B »-fed individuals produced a similar growth rate pattern, but were of different sizes when they reached the 14th instar. If we were to categorize the 14th instar on the basis of biomass or size, we would determine it to be 4th or 5th instar.

In addition to size differences, food quality acts on egg production and may be a strong factor in population dynamics of a given species. As seen from the data present here, Collembola will live and reproduce on both high and low protein foods. But the constituents of these foods may determine their survival and fecundity.

This preliminary work has pointed the way to an avenue of research on Collembola that has been neglected. The step that has to be taken next will be the rearing of Collembola on chemically well-defined diets.

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RÉSUMÉ

INFLUENCE DE L'ALIMENTATION SUR LA CROISSANCE ET LA FÉCONDITÉ D'*O. JUSTI*

Pendant une période de 100 jours, des élevages de *Onychiurus justi* (Denis) ont été maintenus à 70°F et 100 p. 100 H. R. Trois différentes sources de nourriture ont été utilisées : levure, nourriture riche en protéine et pauvre en protéine. Une quatrième série a été maintenue dans les mêmes conditions, mais sans nourriture. Les résultats des observations ont démontré que les divers régimes ont différents effets sur la croissance, la durée des stades, la mortalité, la fécondité et la taille des animaux. Ces différences sont présentées.

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